IFWO

RAW SEQUENCE LISTING DATE: 08/31/2004
PATENT APPLICATION: **US/10/757,909** TIME: 13:36:49

Input Set : **N:\Crf3\RULE60\10757909.raw.txt**
Output Set: **N:\CRF4\08312004\J757909.raw**

<110> APPLICANT: DeBonte, Lorin R.
Fan, Zhegong
Miao, Guo-Hua
<120> TITLE OF INVENTION: FATTY ACID DESATURASES AND MUTANT SEQUENCES THEREOF
<130> FILE REFERENCE: 07148-063003
<140> CURRENT APPLICATION NUMBER: US/10/757,909
<141> CURRENT FILING DATE: 2004-01-15
<150> PRIOR APPLICATION NUMBER: US/09/771,904
<151> PRIOR FILING DATE: 2001-01-29
<150> PRIOR APPLICATION NUMBER: US 08/874,109
<151> PRIOR FILING DATE: 1997-06-12
<160> NUMBER OF SEQ ID NOS: 70
<170> SOFTWARE: FastSEQ for Windows Version 4.0
<210> SEQ ID NO: 1
<211> LENGTH: 1155
<212> TYPE: DNA
<213> ORGANISM: Brassica napus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)...(1152)
<223> OTHER INFORMATION: Wild type Fad2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 205
<223> OTHER INFORMATION: n = a, g, c, or t/u
<400> SEQUENCE: 1

```
atg ggt gca ggt gga aga atg caa gtg tct cct ccc tcc aag aag tct       48
Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser
  1               5                  10                  15

gaa acc gac acc atc aag cgc gta ccc tgc gag aca ccg ccc ttc act       96
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
             20                  25                  30

gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg      144
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
         35                  40                  45

atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc      192
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
     50                  55                  60

tgc ttc tac tac ntc gcc acc act tac ttc cct ctc ctc cct cac cct      240
Cys Phe Tyr Tyr Xaa Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
 65                  70                  75                  80

ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc caa ggg tgc gtc      288
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
```

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Input Set : N:\Crf3\RULE60\10757909.raw.txt
Output Set: N:\CRF4\08312004\J757909.raw

```
                    85                  90                  95
cta acc ggc gtc tgg gtc ata gcc cac gaa tgc ggc cac cac gcc ttc      336
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
            100                 105                 110
agc gac tac cag tgg ctt gac gac acc gtc ggt ctc atc ttc cac tcc      384
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
        115                 120                 125
ttc ctc ctc gtc cct tac ttc tcc tgg aag tac agt cat cgc agc cac      432
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His
    130                 135                 140
cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag      480
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145                 150                 155                 160
aag aag tca gac atc aag tgg tac ggc aag tac ctc aac aac cct ttg      528
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
                165                 170                 175
gga cgc acc gtg atg tta acg gtt cag ttc act ctc ggc tgg ccg ttg      576
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
            180                 185                 190
tac tta gcc ttc aac gtc tcg gga aga cct tac gac ggc ggc ttc cgt      624
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Arg
        195                 200                 205
tgc cat ttc cac ccc aac gct ccc atc tac aac gac cgc gag cgt ctc      672
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
    210                 215                 220
cag ata tac atc tcc gac gct ggc atc ctc gcc gtc tgc tac ggt ctc      720
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225                 230                 235                 240
ttc cgt tac gcc gcc ggc cag gga gtg gcc tcg atg gtc tgc ttc tac      768
Phe Arg Tyr Ala Ala Gly Gln Gly Val Ala Ser Met Val Cys Phe Tyr
                245                 250                 255
gga gtc ccg ctt ctg att gtc aat ggt ttc ctc gtg ttg atc act tac      816
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
            260                 265                 270
ttg cag cac acg cat cct tcc ctg cct cac tac gat tcg tcc gag tgg      864
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
        275                 280                 285
gat tgg ttc agg gga gct ttg gct acc gtt gac aga gac tac gga atc      912
Asp Trp Phe Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
    290                 295                 300
ttg aac aag gtc ttc cac aat att acc gac acg cac gtg gcc cat cat      960
Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
305                 310                 315                 320
ccg ttc tcc acg atg ccg cat tat cac gcg atg gaa gct acc aag gcg     1008
Pro Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
                325                 330                 335
ata aag ccg ata ctg gga gag tat tat cag ttc gat ggg acg ccg gtg     1056
Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
            340                 345                 350
```

RAW SEQUENCE LISTING DATE: 08/31/2004
PATENT APPLICATION: US/10/757,909 TIME: 13:36:49

Input Set : N:\Crf3\RULE60\10757909.raw.txt
Output Set: N:\CRF4\08312004\J757909.raw

```
gtt aag gcg atg tgg agg gag gcg aag gag tgt atc tat gtg gaa ccg     1104
Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
        355                 360                 365
gac agg caa ggt gag aag aaa ggt gtg ttc tgg tac aac aat aag tta     1152
Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
    370                 375                 380
 tga                                                               1155
<210> SEQ ID NO: 2
<211> LENGTH: 384
<212> TYPE: PRT
<213> ORGANISM: Brassica napus
<220> FEATURE:
<223> OTHER INFORMATION: Xaa = Phe, Leu, Ile, or Val
<400> SEQUENCE: 2
Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser
  1               5                  10                  15
Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
             20                  25                  30
Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
         35                  40                  45
Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
     50                  55                  60
Cys Phe Tyr Tyr Xaa Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
 65                  70                  75                  80
Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
                 85                  90                  95
Leu Thr Gly Val Trp Val Ile Ala His Glu Cys Gly His His Ala Phe
            100                 105                 110
Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
        115                 120                 125
Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His
    130                 135                 140
His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
145                 150                 155                 160
Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
                165                 170                 175
Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
            180                 185                 190
Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Arg
        195                 200                 205
Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
    210                 215                 220
Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
225                 230                 235                 240
Phe Arg Tyr Ala Ala Gly Gln Gly Val Ala Ser Met Val Cys Phe Tyr
                245                 250                 255
Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
            260                 265                 270
Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
```

RAW SEQUENCE LISTING DATE: 08/31/2004
PATENT APPLICATION: **US/10/757,909** TIME: 13:36:49

Input Set : **N:\Crf3\RULE60\10757909.raw.txt**
Output Set: **N:\CRF4\08312004\J757909.raw**

```
144                275                 280                 285
145       Asp Trp Phe Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
146           290                 295                 300
147       Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
148       305                 310                 315                 320
149       Pro Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
150                       325                 330                 335
151       Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
152                   340                 345                 350
153       Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
154               355                 360                 365
155       Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
156           370                 375                 380
158 <210> SEQ ID NO: 3
159 <211> LENGTH: 1155
160 <212> TYPE: DNA
161 <213> ORGANISM: Brassica napus
162 <220> FEATURE:
163 <221> NAME/KEY: CDS
164 <222> LOCATION: (1)...(1152)
165 <223> OTHER INFORMATION: G to A transversion mutation at nucleotide 316
166 <220> FEATURE:
167 <221> NAME/KEY: misc_feature
168 <222> LOCATION: 205
169 <223> OTHER INFORMATION: n = a, g, c, or t/u
170 <400> SEQUENCE: 3
171       atg ggt gca ggt gga aga atg caa gtg tct cct ccc tcc aag aag tct      48
172       Met Gly Ala Gly Gly Arg Met Gln Val Ser Pro Pro Ser Lys Lys Ser
173         1               5                  10                  15
174       gaa acc gac acc atc aag cgc gta ccc tgc gag aca ccg ccc ttc act      96
175       Glu Thr Asp Thr Ile Lys Arg Val Pro Cys Glu Thr Pro Pro Phe Thr
176                    20                  25                  30
177       gtc gga gaa ctc aag aaa gca atc cca ccg cac tgt ttc aaa cgc tcg     144
178       Val Gly Glu Leu Lys Lys Ala Ile Pro Pro His Cys Phe Lys Arg Ser
179                35                  40                  45
180       atc cct cgc tct ttc tcc tac ctc atc tgg gac atc atc ata gcc tcc     192
181       Ile Pro Arg Ser Phe Ser Tyr Leu Ile Trp Asp Ile Ile Ile Ala Ser
182            50                  55                  60
-> 183    tgc ttc tac tac ntc gcc acc act tac ttc cct ctc ctc cct cac cct     240
-> 184    Cys Phe Tyr Tyr Xaa Ala Thr Thr Tyr Phe Pro Leu Leu Pro His Pro
185        65                  70                  75                  80
186       ctc tcc tac ttc gcc tgg cct ctc tac tgg gcc tgc caa ggg tgc gtc     288
187       Leu Ser Tyr Phe Ala Trp Pro Leu Tyr Trp Ala Cys Gln Gly Cys Val
188                     85                  90                  95
189       cta acc ggc gtc tgg gtc ata gcc cac aag tgc ggc cac cac gcc ttc     336
190       Leu Thr Gly Val Trp Val Ile Ala His Lys Cys Gly His His Ala Phe
191                100                 105                 110
192       agc gac tac cag tgg ctt gac gac acc gtc ggt ctc atc ttc cac tcc     384
193       Ser Asp Tyr Gln Trp Leu Asp Asp Thr Val Gly Leu Ile Phe His Ser
```

RAW SEQUENCE LISTING DATE: 08/31/2004
PATENT APPLICATION: US/10/757,909 TIME: 13:36:49

Input Set : N:\Crf3\RULE60\10757909.raw.txt
Output Set: N:\CRF4\08312004\J757909.raw

```
194                 115                 120                 125
195       ttc ctc ctc gtc cct tac ttc tcc tgg aag tac agt cat cgc agc cac      432
196       Phe Leu Leu Val Pro Tyr Phe Ser Trp Lys Tyr Ser His Arg Ser His
197           130                 135                 140
198       cat tcc aac act ggc tcc ctc gag aga gac gaa gtg ttt gtc ccc aag      480
199       His Ser Asn Thr Gly Ser Leu Glu Arg Asp Glu Val Phe Val Pro Lys
200       145                 150                 155                 160
201       aag aag tca gac atc aag tgg tac ggc aag tac ctc aac aac cct ttg      528
202       Lys Lys Ser Asp Ile Lys Trp Tyr Gly Lys Tyr Leu Asn Asn Pro Leu
203                       165                 170                 175
204       gga cgc acc gtg atg tta acg gtt cag ttc act ctc ggc tgg ccg ttg      576
205       Gly Arg Thr Val Met Leu Thr Val Gln Phe Thr Leu Gly Trp Pro Leu
206                   180                 185                 190
207       tac tta gcc ttc aac gtc tcg gga aga cct tac gac ggc ggc ttc cgt      624
208       Tyr Leu Ala Phe Asn Val Ser Gly Arg Pro Tyr Asp Gly Gly Phe Arg
209               195                 200                 205
210       tgc cat ttc cac ccc aac gct ccc atc tac aac gac cgc gag cgt ctc      672
211       Cys His Phe His Pro Asn Ala Pro Ile Tyr Asn Asp Arg Glu Arg Leu
212           210                 215                 220
213       cag ata tac atc tcc gac gct ggc atc ctc gcc gtc tgc tac ggt ctc      720
214       Gln Ile Tyr Ile Ser Asp Ala Gly Ile Leu Ala Val Cys Tyr Gly Leu
215       225                 230                 235                 240
216       ttc cgt tac gcc gcc ggc cag gga gtg gcc tcg atg gtc tgc ttc tac      768
217       Phe Arg Tyr Ala Ala Gly Gln Gly Val Ala Ser Met Val Cys Phe Tyr
218                       245                 250                 255
219       gga gtc ccg ctt ctg att gtc aat ggt ttc ctc gtg ttg atc act tac      816
220       Gly Val Pro Leu Leu Ile Val Asn Gly Phe Leu Val Leu Ile Thr Tyr
221                   260                 265                 270
222       ttg cag cac acg cat cct tcc ctg cct cac tac gat tcg tcc gag tgg      864
223       Leu Gln His Thr His Pro Ser Leu Pro His Tyr Asp Ser Ser Glu Trp
224               275                 280                 285
225       gat tgg ttc agg gga gct ttg gct acc gtt gac aga gac tac gga atc      912
226       Asp Trp Phe Arg Gly Ala Leu Ala Thr Val Asp Arg Asp Tyr Gly Ile
227           290                 295                 300
228       ttg aac aag gtc ttc cac aat att acc gac acg cac gtg gcc cat cat      960
229       Leu Asn Lys Val Phe His Asn Ile Thr Asp Thr His Val Ala His His
230       305                 310                 315                 320
231       ccg ttc tcc acg atg ccg cat tat cac gcg atg gaa gct acc aag gcg     1008
232       Pro Phe Ser Thr Met Pro His Tyr His Ala Met Glu Ala Thr Lys Ala
233                       325                 330                 335
234       ata aag ccg ata ctg gga gag tat tat cag ttc gat ggg acg ccg gtg     1056
235       Ile Lys Pro Ile Leu Gly Glu Tyr Tyr Gln Phe Asp Gly Thr Pro Val
236                   340                 345                 350
237       gtt aag gcg atg tgg agg gag gcg aag gag tgt atc tat gtg gaa ccg     1104
238       Val Lys Ala Met Trp Arg Glu Ala Lys Glu Cys Ile Tyr Val Glu Pro
239               355                 360                 365
240       gac agg caa ggt gag aag aaa ggt gtg ttc tgg tac aac aat aag tta     1152
241       Asp Arg Gln Gly Glu Lys Lys Gly Val Phe Trp Tyr Asn Asn Lys Leu
242           370                 375                 380
```

RAW SEQUENCE LISTING ERROR SUMMARY DATE: 08/31/2004
PATENT APPLICATION: **US/10/757,909** TIME: 13:36:50

Input Set : **N:\Crf3\RULE60\10757909.raw.txt**
Output Set: **N:\CRF4\08312004\J757909.raw**

Please Note:
Use of n and/or Xaa have been detected in the Sequence Listing. Please review the Sequence Listing to ensure that a corresponding explanation is presented in the <220> to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:1; N Pos. 205
Seq#:1; Xaa Pos. 69
Seq#:2; Xaa Pos. 69
Seq#:3; N Pos. 205
Seq#:3; Xaa Pos. 69
Seq#:4; Xaa Pos. 69

VARIABLE LOCATION SUMMARY DATE: 08/31/2004
PATENT APPLICATION: **US/10/757,909** TIME: 13:36:50

Input Set : **N:\Crf3\RULE60\10757909.raw.txt**
Output Set: **N:\CRF4\08312004\J757909.raw**

Use of n's or Xaa's(NEW RULES):
Use of n's and/or Xaa's have been detected in the Sequence Listing.
Use of <220> to <223> is MANDATORY if n's or Xaa's are present.
in <220> to <223> section, please explain location of n or Xaa, and which residue n or Xaa represents.

Seq#:1; N Pos. 205
Seq#:1; Xaa Pos. 69
Seq#:2; Xaa Pos. 69
Seq#:3; N Pos. 205
Seq#:3; Xaa Pos. 69
Seq#:4; Xaa Pos. 69

VERIFICATION SUMMARY DATE: 08/31/2004
PATENT APPLICATION: **US/10/757,909** TIME: 13:36:50

Input Set : **N:\Crf3\RULE60\10757909.raw.txt**
Output Set: **N:\CRF4\08312004\J757909.raw**

L:40 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:1 after pos.:192
L:41 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:1 after pos.:240
L:117 M:258 W: Mandatory Feature missing, <221> Tag not found for SEQ ID#:2
L:117 M:258 W: Mandatory Feature missing, <222> Tag not found for SEQ ID#:2
L:117 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:2 after pos.:64
L:183 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:3 after pos.:192
L:184 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:3 after pos.:240
L:260 M:258 W: Mandatory Feature missing, <221> Tag not found for SEQ ID#:4
L:260 M:258 W: Mandatory Feature missing, <222> Tag not found for SEQ ID#:4
L:260 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:4 after pos.:64